INTRODUCTION

Bacteria were very small and harmful organism, some bacteria could cause various types of infectious diseases such as typhoid [1], common cold [2], influenza [3], pneumonia [4], malaria [5], amebiasis [6], syphilis and AIDS [7]. Bacteria may be innate resistant or acquire resistance to one or few classes of antimicrobial agents. Bacterial resistance to antimicrobial drugs was an increased health and economic problem [8] so, developed a new antibacterial agent was so important. An antibacterial agent from the natural material like curcumin has been developed [9-11].

Curcumin-related studies were increasingly evolving with more modification of curcumin structure and its activity test as antibacterial. Some studies related to curcumin modification as an antibacterial include curcumin modification into mono-carbonyl analogue curcumin [12], curcumin bioconjugate [13], heterocyclic moiety curcumin [14], Curcuminaniline Functionalized Nanoform [15]. Most of the curcumin modification reported could enhance the antibacterial activity.

One of the other curcumin modification compounds was 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one with the molecular formula C_{21}H_{22}O_{5} (fig. 1). The 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one compound has been successfully synthesized with aldol condensation method [16,17]. Activity tested of 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one as antioxidants showed better ability than curcumin. The anti-inflammatory activity of 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one has been performed and indicated a better than curcumin [18]. 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one was also expected to have a good antibacterial activity. So, this study aimed to test antibacterial activity of 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one using in silico and in vitro evaluation. In vitro evaluation were used for several types of bacteria on a gram-positive group (Staphylococcus aureus, Staphylococcus epidermidis), and gram-negative group (Escherichia coli, Salmonella Typhi). The in silico evaluation was performed using molecular docking on receptor PDB ID 3MZD. This antibacterial activity test refers to the inhibition of the D-Alanil-D-Alanin transport enzyme (D-Alanil-D-Alanin decarboxylase (DACA)) on peptidoglycan formation on the bacterial cell wall, so in this study used β-lactam group antibiotic such as amoxicillin and cefadroxil as a positive control. The β-lactam group antibiotics were produced most widely and were used as antibacterial drugs in the world, and have been used since the initial appearance of clinical trials in 1941 [19, 20].

MATERIALS AND METHODS

Chemicals

The following chemicals and materials were used: Ethyl Vanillin (3’-ethoxy-4’-hydroxybenzaldehyde) (Sigma Aldrich), glacial acetic acid (Mallincordt Chemical), Chloric acid (Mallincordt Chemical), acetone (Mallincordt Chemical), chloroform (Mallincordt Chemical), ethyl acetate (Mallincordt Chemical), methanol (Mallincordt Chemical), metabisulfit (Mallincordt Chemical), 96% ethanol (Mallincordt Chemical), aquadest. All other ingredients used were of analytical grade.
Culture media

Nutrient agar (NA), peptone broth, the test bacteria used in this study were a clinical isolate of Staphylococcus aureus (ATCC 6538), Staphylococcus epidermidis (ATCC 12228), Escherichia coli (ATCC 25922), and Salmonella thypi (ATCC 14028) supplied by the Department of Microbiology Medical Faculty, University of Indonesia.

Instruments

Allihn condensor, Flat-bottom flask, magnetic stirrer, volume pipette, micropipette Eppendorf, chromatograph vessel, Silica gel plate GF254, glass tools (pyrex), filter paper, Spatula, parchment paper, petri dish Spectrophotometer ultraviolet-Visible (Shimadzu, UV 1601), Spectrophotometer Fourier Transform-Infra Red (Shimadzu FT-IR 6400S), Spectrophotometer Nuclear Magnetic Resonance (NMR) (JEOL, JNM ECA-500), Gas Chromatography-Mass Spectrometer (Agilent Technologies 6890 Gas Chromatograph and 5972 mass selective detector), Thin Layer Chromatography (TLC), Densitometer (Camag TLC Scanner 3), Melting range finder (BUCHI 540), Analytical scales (Mettler TTS), Stir Plate and magnetic stirrer, Allihn condensor, capillary tube, Oven Microwave (Cevilla CMG 003.21L).

Experimental method

Synthesis of 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one was carried out using aldol condensation reaction. Methanol and acetone in the flat flask connected to Allihn condenser at room temperature were stirred using magnetic stirrer for 5 min, ethyl vanillin and concentrated HCl was added until pH value of 2-3, stir for 2h at 500 rpm. exposure to microwave radiation over time, observed using Thin Layer Chromatography (TLC) and densitometry method in Silica gel GF254 was eluted with chloroform-ethyl acetate (9:1). The yield as aromatic odor, yellow and brownish green color, m. p: (95.5-96.4 °C), FTIR (KBr): 1676,69 cm⁻¹ (C = O), 1578.63 cm⁻¹ (C = C is conjugated with the aromatic core), 3500-2500 cm⁻¹ (O-H group), 2979.02 and 2931.60 cm⁻¹ (C-H), 654.79 and 838.98 cm⁻¹ (C=H aromatic ring substitution). Thus, the aldehyde group in ethyl vanillin had shifted to the keto group in the synthesis compounds.¹³C NMR and ¹H NMR Analysis chemical shift signal at δ 31.δ 4.16 (quartet), while δ 5.94 (singlet) shows the chemical compound has 11 protons. In regions of high magnetic fields and δ 64.8 indicate the presence of carbon at O=C=H group.

The 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one compound was prepared based on the α, β disconnect analysis on EHP. Preparations of 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one and amoxicillin and cefadroxil were screened in vitro for determined Minimum Inhibitory Concentration (MIC) using the broth dilution method. Staphylococcus aureus (ATCC 6538), Staphylococcus epidermidis (ATCC 12228), Escherichia coli (ATCC 25922), and Salmonella thypi (ATCC 14028). Bacterial strains were cultivated separately in nutrient agar plates. The bacterial inoculums (0.25 optical density (OD)) was prepared and incubated for 24h [23]. The 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one, prepared in serially concentration 0,30; 0,25; 0,20; 0,175; 0,15; 0,125; 0,10 mg/L. The amoxicillin and cefadroxil concentration prepared in serially concentration 32; 16; 8; 4; 2; 1; 0,5; 0,25 mg/L. All of them prepared in test tubes. The bacterial inoculums were added each 0.5 ml into the test tubes then incubated at 35–37 °C for 18-24 h. The Minimum Inhibitory Concentration (MIC) for bacteria was determined as the lowest concentration of the compound inhibiting the visual growth of the test cultures on the agar plates.

Measurement of the diameter of inhibition zone

The diameter of inhibition zone was performed using Kirby-Bauer disc diffusion method. The bacterial inoculums (0.25 optical density (OD)) inserted into each sterile petri dish 0.5 ml, added 15–20 ml of Nutrient Agar (NA). Then homogenized and solidified the bacteria suspension and Nutrient Agar (NA) medium at room temperature for 15-30 min. After the solidified, inserted solution of EHP with various concentrations into the petri dish. Then incubated at 35–37 °C for 24h [23]. Measured clear zone formed with units of millimetres (mm). The same procedure as determined by the diameter of the inhibitory zone of amoxicillin and cefadroxil.

RESULTS AND DISCUSSION

The synthesis of 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one compound was prepared based on the α, β disconnect analysis on target molecules by methods developed by Stuart Warren (2008) (fig. 2) [24].

![Fig. 2: α, β disconnect analysis on EHP](image-url)
aldehyde molecule previously undergoes a protonation of its carbonyl group, producing a β-hydroxy carbonyl compound. Beginning with a nucleophile attack on carbon atoms from carbon-oxygen double bonds, it occurs because carbon has a positive charge. The adduction reaction takes place, followed by a dehydration process and produces a carbonyl α, β unsaturated. The FTIR, 13C NMR, 1H NMR and elemental analysis were consistent with the assigned structure.

Validation of molecular docking protocol was performed to ensure that the receptor was suitable for use in molecular docking process. The validation process obtained Root Mean Square Deviation (RMSD) value of 1.9553 Å. Root Mean Square Deviation (RMSD) value less than 2 Å indicated that the docking protocol with PDB ID 3MZD receptor was feasible to be used for docking process of 1,5-bis (3'-ethoxy-4'-hydroxyphenyl)-1,4-pentadiene-3-one as an antibacterial agent and the alignment between the reference ligand and the conformation of the docked ligand was very good (fig. 4) [25].

Docking process of 1,5-bis (3'-ethoxy-4'-hydroxyphenyl)-1,4-pentadiene-3-one with PDB ID 3MZD receptor obtained docking score of -91.5305, it was much lower than the docking score of cloxacinil acyl which is native ligand (-81.9278). It was possible that 1,5-bis (3'-ethoxy-4'-hydroxyphenyl)-1,4-pentadiene-3-one becomes potential antibacterial compounds. Comparing with amoxicillin and cefadroxil, the antibacterial activity of the 1,5-bis (3'-ethoxy-4'-hydroxyphenyl)-1,4-pentadiene-3-one is better, it showed by docking score of amoxicillin ( -87.6313) and the cefadroxil (-85.4935) greater than docking score of 1,5-bis (3'-ethoxy-4'-hydroxyphenyl)-1,4-pentadiene-3-one. The lower docking score was the better stability level between ligand and receptor so the stronger bonds will be formed. The negative value of binding energy change (ΔG) reveals that the binding process is spontaneous it could fit well in the binding pocket receptor forming most stable drug receptor energetically [26]. Larger the negative value of binding energy, greater the chemical be accepted as a drug [27]. The position of ligands in the binding pocket of PDB ID 3MZD receptor showed in (fig. 5), hydrophobic site show in green areas and hydrogen bond in purple areas.
The amino acid residues on the active site of the PDB ID 3MZD receptor that formed the hydrogen bond with the cloxacillin acyl there were 4 i.e ALA171, ARG174, ILE173, and LEU172. The oxygen on carbonyl group of cloxacillin acyl ligand has bonded with hydrogen in the amino group of LEU172 (2.2 Å) and ALA171 (2 Å) while the other oxygen on carbonyl group has bonded with the hydrogen atoms on the amino group of ILE173 (1.7 Å) and ARG174 (1.8 Å). Amino acid residues in the PDB ID 3MZD receptor binding sites that formed hydrogen bonds with 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one were included LEU167 and ILE173. The oxygen on hydroxy group of 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one has bonded with hydrogen in the amino group of LEU167 (2.5 Å) while the oxygen on carbonyl group has bonded with hydrogen in amino group of ILE173 (1.7 Å). In amoxicillin hydrogen bonds were formed between oxygen on carbonyl group with hydrogen on amino group of LEU172 (1.6 Å) while in cefadroxil, hydrogen bonds were formed by hydrogen of the methyl group with the oxygen on LEU167 (2.4 Å) and the oxygen on carbonyl group with the hydrogen on the amino group of ILE173 (1.7 Å) and SER227 (2.2 Å) (fig. 6). The amino acid residues affected the bond on the PDB ID 3MZD receptor, the interaction of the ligand and PDB ID 3MZD receptor may occur due to hydrogen bonding, Van Der Waals bonding, and electrostatic interactions [23].

### Table 1: Minimum inhibitory concentration (ppm)

<table>
<thead>
<tr>
<th>Test compound</th>
<th>Staphylococcus aureus (ATCC 6538)</th>
<th>Staphylococcus epidermidis (ATCC 12228)</th>
<th>Escherichia coli (ATCC 25922)</th>
<th>Salmonella thyphi (ATCC 14028)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EHP</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Cefadroxil</td>
<td>1</td>
<td>8</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>
In vitro evaluation of 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one as antibacterial

Minimum inhibitory concentrations (MIC)

Evaluation of Minimum Inhibitory Concentration (MIC) aimed to determine the lowest concentration of a test compound in inhibiting the growth of bacteria (table 1).

The 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one was inhibited test bacteria with a lower concentration than amoxicillin and cefadroxil. It’s could inhibit test bacteria at minimum inhibitory concentrations (MIC) of 0.15 ppm for all test bacteria. It was indicated that the concentration of 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one has bacteriostatic properties. While the Minimum Inhibitory Concentration (MIC) of amoxicillin obtained at 2 ppm for Staphylococcus aureus (ATCC 6538), 4 ppm for Staphylococcus epidermidis (ATCC 12228), 2 ppm for Escherichia coli (ATCC 25922), and 1 ppm for Salmonella thyphi (ATCC 14028). The minimum inhibitory concentrations of cefadroxil obtained at 1 ppm for Staphylococcus aureus (ATCC 6538), 8 ppm for Staphylococcus epidermidis (ATCC 12228), 4 ppm for Escherichia coli (ATCC 25922), and 2 ppm for Salmonella thyphi (ATCC 14028). Mechanism of amoxicillin and cephalosporins as antibiotics inhibited the synthesis of microbial cell walls, both active antibiotics against gram-positive and gram-negative, but the antimicrobial spectra of each derivative were varied [11]. The differences of Minimum Inhibitory Concentration (MIC) value of both antibiotics caused by amoxicillin is the prototype of aminopenicillin group with wide-spectrum that can inhibit bacteria from the gram-positive and gram-negative group. While cefadroxil was the first generation of cephalosporins, the cephalosporin group was broad-spectrum but the first class of cephalosporins exhibits an antimicrobial spectrum that was particularly active against gram-positive bacteria. Minimum Inhibitory Concentration (MIC) value on gram-positive bacteria (Staphylococcus epidermidis), cefadroxil has a higher than gram-positive bacteria (Staphylococcus aureus). Increased of Minimum Inhibitory Concentration (MIC) of cefadroxil on Staphylococcus epidermidis have been due to bacteria have experienced resistance. Resistance to cefadroxil due to bacteria have formed a β-lactamase enzyme that could degrade the drug, it was caused drug levels that should inhibit cell wall synthesis was reduced, so the effects of antibiotic to bacteria were reduced. The 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one have the same antibacterial activity in both gram-positive and gram-negative bacteria with the same Minimum Inhibitory Concentration (MIC) value of 0.15 ppm, even in staphylococcus aureus and escherichia coli, in previously reported the 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one have better antibacterial activities than antibacterial activities of curcumin [17,18]. This indicated that 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one has a broad spectrum as antibacterial, this was related to the lipophilicity of 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one. Substituted of the methoxy group on curcumin with ethoxy groups have made 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one have higher lipophilicity than curcumin, amoxicillin, and cefadroxil, in addition to steric properties was also greater. The large lipophilicity of 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one has allows 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one to diffuse in gram-negative bacteria cell walls that composed of lipopolysaccharides, lipoproteins, and phospholipids. The steric properties have facilitated 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one to put physical pressure on gram-positive cell walls [19, 20].

The diameter of inhibition zone

Determination of diameter of inhibition zone aimed to determine the sensitivity of bacterial testing of 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one characterized by the presence of clear zones around the disc (fig 7). The tests were performed on 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one, amoxicillin and cefadroxil. To determine the diameter of inhibition zone of 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one, amoxicillin and cefadroxil to the test bacteria were tested by disc diffusion method with some predetermined concentration. The clear zone of 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one to the test bacteria were average of 11 mm for all test bacteria. The diameter of Inhibition Zone value of 10-16 mm indicates moderate activity as antibacterial [23]. While the diameter of inhibition zone of amoxicillin and cefadroxil showed the varied results of each test bacteria, this difference occurs because of the antimicrobial spectra of each derivative varies although the mechanism of action was the same i.e. inhibiting cell wall growth (table 2).
**Table 2: Diameter of inhibition zone (mm)**

<table>
<thead>
<tr>
<th>Test compound</th>
<th>Staphylococcus aureus (ATCC 6538)</th>
<th>Staphylococcus epidermidis (ATCC 12228)</th>
<th>Escherichia coli (ATCC 25922)</th>
<th>Salmonella typhi (ATCC 14028)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one</td>
<td>11.27±0.31</td>
<td>11.35±0.39</td>
<td>11.25±0.33</td>
<td>11.05±0.45</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>8.22±0.25</td>
<td>8.07±0.38</td>
<td>7.93±0.32</td>
<td>8.18±0.30</td>
</tr>
<tr>
<td>Cefadroxil</td>
<td>8.33±0.33</td>
<td>6.7±0.17</td>
<td>7.52±0.28</td>
<td>8.45±0.18</td>
</tr>
</tbody>
</table>

*Means±SD, n = 3

The diameter of inhibition zone that obtained of 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one was reproduced against test bacteria compared with β-lactam group antibiotics have been analyzed using statistical data processing program. Statistical analysis was done to determine normality and homogeneity of data. The test results obtained normal distributed and homogeneously distributed with p-value>0.05. The one-way ANOVA parametric test was done to determine the difference of diameter of inhibition zone of 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one compared to the β-lactam group antibiotics. It was obtained significance value of p = 0.000 (p<0.05). It meant there was a significant difference between 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one and β-lactam group antibiotic [28]. From the results obtained that the 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one has a better antibacterial activity compared with β-lactam group antibiotics. This difference may occur because 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one has a stronger mechanism of action as inhibitors of cell wall synthesis. The cell wall of the bacterial class contains thin peptidoglycan and the remaining proteins, lipopolysaccharides, and lipoproteins, so the 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one could disrupt the synthesis of cell wall formation and could cause cell lysis, then cause death in microbial cells.

**CONCLUSION**

The 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one as curcumin in analog compounds have biological activity as antibacterial. The antibacterial activity of 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one was stronger than the β-lactam group antibiotics both amoxicillin and cefadroxil. Based on Minimum Inhibitory Concentration (MIC) and diameter of inhibition zone, 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one has strong antibacterial activity in both gram-positive and gram-negative bacteria. An in vitro evaluation was in line with in silico evaluation that showed the 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one has the lowest docking score.

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**AUTHORS CONTRIBUTIONS**

All of the authors designed, directed the project and contributions on wrote the article; on experiment work, Purwangga performed the in vitro experiment, Mumpuni performed the synthesis and characterized 1,5-bis (3’-ethoxy-4’-hydroxyphenyl)-1,4-pentadiene-3-one, Mulatsari performed the in silico experiment.

**CONFLICTS OF INTERESTS**

All authors have none to declare.

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